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Pharmacological Analysis of the Novel, Rapid, and Potent Inactivation of the Human 5-Hydroxytryptamine$_7$ Receptor by Risperidone, 9-OH-Risperidone, and Other Inactivating Antagonists

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ABSTRACT

In a previous publication, using human 5-hydroxytryptamine$_7$ (h5-HT$_7$) receptor-expressing human embryonic kidney (HEK) 293 cells, we reported the rapid, potent inactivation of the h5-HT$_7$ receptor stimulation of cAMP production by three antagonists: risperidone, 9-OH-risperidone, and methiothepin (Smith et al., 2006). To better understand the drug-receptor interaction producing the inactivation, we 1) expanded the list of inactivating drugs, 2) determined the inactivating potencies and efficacies by performing concentration-response experiments, and 3) determined the potencies and efficacies of the inactivators as irreversible binding site inhibitors. Three new drugs were found to fully inactivate the h5-HT$_7$ receptor: lisuride, bromocryptine, and metergoline. As inactivators, these drugs displayed potencies of 1, 80, and 321 nM, respectively. Pretreatment of 5-HT$_7$-expressing HEK cells with increasing concentrations of the inactivating drugs risperidone, 9-OH-risperidone, methiothepin, lisuride, bromocriptine, and metergoline potently inhibited radiolabeling of the h5-HT$_7$ receptor, with IC$_{50}$ values of 9, 5.5, 152, 3, 73, and 10 nM, respectively. We were surprised to find that maximal concentrations of risperidone and 9-OH-risperidone inhibited only 50% of the radiolabeling of h5-HT$_7$ receptors. These results indicate that risperidone and 9-OH risperidone may be producing 5-HT$_7$ receptor inactivation by different mechanisms than lisuride, bromocryptine, metergoline, and methiothepin. These results are not interpretable using the conventional model of G-protein-coupled receptor function. The complex seems capable of assuming a stable inactive conformation as a result of the interaction of certain antagonists. The rapid, potent inactivation of the receptor-G-protein complex by antagonists implies a constitutive, pre-existing complex between the h5-HT$_7$ receptor and a G-protein.

The 5-HT$_7$ receptor is 1 of 13 5-HT receptors expressed in mammalian tissues (Teitler and Herrick-Davis, 1994; Gerhardt and van Heerikhuizen, 1997; Hoyer and Martin, 1997; Raymond et al., 2001; Hoyer et al., 2002; Kroeze et al., 2002). It was discovered through homology cloning and is expressed in various areas of the human brain and in peripheral tissues, including important blood vessels in the cerebral vasculature (Bard et al., 1993; Lovenberg et al., 1993; Shen et al., 1993; Teitler and Herrick-Davis, 1994; Hedlund and Sutcliffe, 2004). 5-HT$_7$ receptor antagonists are being developed for possible use in various clinical conditions, including migraine (Terron, 1997), sleep (Lovenberg et al., 1993), psychosis (Bard et al., 1993; Lovenberg et al., 1993; Shen et al., 1993), and depression (Bard et al., 1993; Lovenberg et al., 1993; Shen et al., 1993; Hedlund and Sutcliffe, 2004).

Risperidone is a highly prescribed atypical antipsychotic drug (Bhana and Spencer, 2000; Gilbody et al., 2000; Green, 2000; Love and Nelson, 2000; Schneider et al., 2006). It is one of a group of drugs believed to initiate their effects through interactions with the D$_2$ dopamine and 5-HT$_{2A}$ serotonin receptors (Meltzer et al., 1989; Roth et al., 1994). These interactions have been shown to be classic competitive antagonist interactions (Roth et al., 1994; Smith et al., 2006). We reported recently that risperidone, 9-OH-risperidone, which is the active metabolite of risperidone (Ereshefsky and Lacombe, 1993; Borison et al., 1994; Spina et al., 2001), and methiothepin, a classic, nonselective 5-HT receptor antagonist, produce a unique effect on cells expressing the h5-HT$_7$ receptor (Smith et al., 2006). Pretreatment with these three

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ABBREVIATIONS: 5-HT$_7$, 5-hydroxytryptamine$_7$; HEK, human embryonic kidney; GPCR, G-protein-coupled receptor; LSD, *d*-lysergic acid diethylamide; SB269970, (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol hydrochloride; ICI169369, 2-(2-dimethylamino ethylthio)-3-phenyl quinoline; BIIE0246, *N*-[(1*S*)-4-[(aminoiminomethyl)amino]-1-[[[2-(3,5-dioxo-1,2-diphenyl-1,2,4-triazolidin-4-yl)ethyl]amino]carbonyl]butyl]-1-[2-[4-(6,11-dihydro-6-oxo-5H-dibenz[*b*,*e*]azepin-11-yl)-1-piperazinyl]-2-oxoethyl]-cyclopentaneacetamide formate.

drugs at low concentrations for a short time (30 min) produces a long-lasting inactivation of the h5-HT$_7$ receptor. [^{3}H]Risperidone binding studies indicated that this effect is due to an irreversible complex formed between risperidone and the h5-HT$_7$ receptor. It is presumed that a similar mechanism is responsible for the effects of 9-OH-risperidone and methiothepin, although this can only be indirectly demonstrated, because radiolabeled forms of these latter two drugs are not available. It is interesting that exposing membrane homogenates prepared from h5-HT$_7$-expressing cells to risperidone or 9-OH-risperidone did not produce an irreversible occlusion of the binding site (Smith et al., 2006). These results indicate that the receptor must be in the cellular environment to irreversibly bind these drugs.

Nine drugs studied at the same time as risperidone, 9-OH-risperidone, and methiothepin did not display the inactivating properties (Smith et al., 2006). To more fully examine the pharmacological properties of drugs that promote the irreversible interaction with the h5-HT$_7$ receptor, we attempted to expand the list of inactivating drug. Seventeen drugs not studied previously were screened for inactivating properties. These drugs were selected because they had high to moderate affinities for the 5-HT$_7$ receptor, ensuring a drug-receptor interaction at nanomolar to micromolar concentrations (Bard et al., 1993; Lovenberg et al., 1993; Monsma et al., 1993; Ruat et al., 1993; Shen et al., 1993; Roth et al., 1994). Three new inactivating drugs were discovered. The ability of the inactivating drugs to inhibit radioligand binding and 5-HT-stimulated activity was monitored (after drug removal) to determine the relationship between receptor occupancy and inactivating potency. Pretreatment with all six inactivators eliminated all h5-HT$_7$ receptor-mediated activity. Pretreatment with bromocryptine, metergoline, and methiothepin eliminated all radiolabeling of h5-HT$_7$ receptors; lisuride eliminated ~80% of the radiolabeled receptors. However, risperidone and 9-OH-risperidone only maximally inhibited 50% of the radiolabeled h5-HT$_7$ receptors. Taken together, these results indicate the possibility of two different mechanisms of h5-HT$_7$ receptor inactivation among the six inactivating drugs.

Materials and Methods

Homogenate Radioligand Binding. Radioligand binding studies in membrane homogenates were performed as described previously with modifications (Purohit et al., 2005; Smith et al., 2006). HEK cells stably expressing h5-HT$_7$ receptors (100-mm dish; ~100% confluent) were scraped and collected in 50 mM Tris-HCl, 0.5 mM EDTA, and 10 mM $MgSO_4$, pH 7.7 (at 23°C); centrifuged at 14,000*g* for 30 min; homogenized using a Polytron homogenizer (Kinematica, Basel, Switzerland); and centrifuged again at 14,000*g* for 30 min. The membranes were resuspended in HEPES buffer (20 mM HEPES, 2.5 mM $MgSO_4$, and 134 mM NaCl, pH 7.5, 23°C) and 0.1% ascorbic acid. Assays were performed in triplicate 1.0-ml volumes containing 10 μg of membrane protein (which was added last). Assays containing 2 nM [^{3}H]5-HT (24 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) were performed in the absence and presence of 10 μM clozapine to detect the level of available h5-HT$_7$ receptors. Specific binding was generally 85 to 95% of total binding. Tubes were incubated for 30 min at 37°C, filtered, and counted using liquid scintillation. Experimental results were analyzed using Prism 5.0 (GraphPad Software, San Diego, CA). Protein content of the samples was determined with the use of a BCA protein assay kit (Pierce Chemical, Rockford, IL).

Whole-Cell Radioligand Binding. Whole-cell radioligand binding studies were performed as described previously with modifications (Kong et al., 2006; Smith et al., 2006). Cells were lifted using 1 ml/dish diluted trypsin-EDTA, followed by the addition of 5 ml/dish HEPES buffer (see above). Cells were gently centrifuged for 3 min at 330*g*, supernatant was aspirated, and cells were resuspended in HEPES buffer. Cells were pretreated with drug, incubated for 30 min at 37°C, washed three times for 10 min each time with HEPES buffer, resuspended in HEPES buffer, and added to the assay tubes. Assay tubes contained 2 nM [^{3}H]5-HT for competition studies or varying concentrations (0.5–15 nM) for saturation analyses. Clozapine (10 μM) was used to define nonspecific binding. Assay tubes were incubated for 30 min at 37°C, filtered, and counted using liquid scintillation.

cAMP Assay. Total cAMP accumulation was measured using the LANCE cAMP Detection kit (PerkinElmer Life and Analytical Sciences). Cells were lifted using 1 ml/dish Versene followed by the addition of 11 ml of Hanks' buffered saline solution per dish. Cells were centrifuged for 3 min at 330*g*, supernatant was aspirated, and the cells were resuspended in HEPES buffer. Cells were pretreated with drug, incubated 30 min at 37°C, and washed three times for 10 min each time in HEPES buffer. After the third wash, cells were resuspended in stimulation buffer (prepared according to the PerkinElmer LANCE cAMP instruction manual). Cells were counted with a hemacytometer and added to 96-well white opaque plates. The pretreated cells were then exposed to 10 μM 5-HT for 30 min at 23°C. Control experiments demonstrated that this procedure produced no effect on the cells' responsiveness to 5-HT (see *Results*). Time-resolved fluorescence resonance energy transfer was detected by the Victor3 1420 plate reader (PerkinElmer Life and Analytical Sciences).

Results

Figure 1 displays the results of screening 20 drugs for inactivating properties. These drugs were selected based on

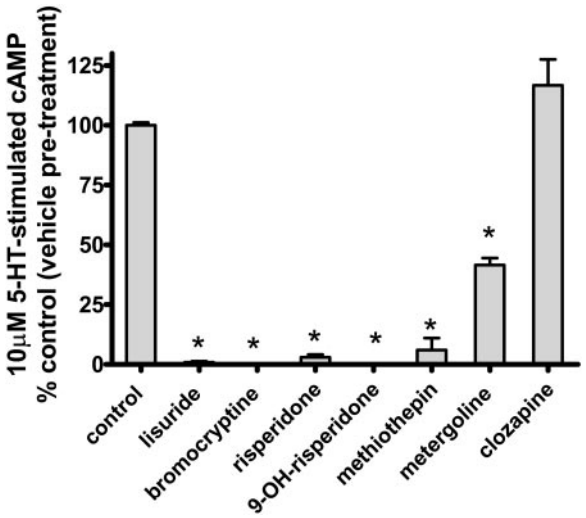

Fig. 1. Effect of drug pretreatment on h5-HT$_7$ receptor-stimulated cAMP production. HEK293 cells stably expressing h5-HT$_7$ receptors were suspended in isotonic buffer and exposed to 1 μM concentrations of drugs for 30 min. Cells were gently pelleted, buffer was replaced, and the cells were exposed to non–drug-containing buffer. This drug washout procedure was repeated three times. Cells were resuspended and assayed for response to 10 μM 5-HT using the LANCE cAMP Detection kit (PerkinElmer) and time-resolved fluorescence resonance energy transfer. The results are the means ± S.E.M. of three independent experiments performed in triplicate. The following drugs (1 μM) displayed no effect on h5-HT$_7$-receptor mediated stimulation of cAMP after washout: ICI169369, tenilapine, cyproheptadine, SB269970, 3-trifluoromethylphenylpiperazine, trifluoperazine, methysergide, ritanserin, loxapine, amoxapine, amitriptyline, and LSD. *, $p < 0.003$ versus control.

preliminary radioligand binding studies indicating that they had high to moderate affinities for the h5-HT_7 receptor. The h5-HT_7 receptor-expressing HEK cells were first exposed to 1 μM drug for 30 min followed by three washouts. The cells were then exposed to 10 μM 5-HT for 30 min. Inactivation was defined as the inability of the cells to produce cAMP in response to the 10 μM 5-HT stimulation. Six of the drugs tested displayed this property: risperidone, 9-OH-risperidone, and methiothepin (characterized previously as inactivators; Smith et al., 2006), and lisuride, bromocryptine, and metergoline, which were not characterized previously. The six drugs tested that had inactivating ability exhibited h5-HT_7 receptor affinities that ranged from 0.4 to 143 nM. Lisuride (0.4 nM), risperidone (2 nM), methiothepin (3 nM), 9-OH-risperidone (10 nM), metergoline (16 nM), and bromocryptine (143 nM) displayed inactivating properties (Figs. 1 and 2). Other drugs tested that did not display inactivating properties are listed with their h5-HT_7 receptor affinities: amoxapine (69 nM); amitriptyline (96 nM); cyproheptadine (24 nM); loxapine (258 nM); mianserin (64 nM); ritanserin (468 nM); the selective 5-HT_7 receptor antagonist SB269970 (2 nM); tenilapine (153 nM); 3-trifluoromethylphenylpiperazine (1624 nM); trifluoperazine (497 nM); the high-affinity 5-HT_2 receptor antagonist ICI169369 (393 nM); clozapine (30 nM); LSD (3 nM); and methysergide (32 nM). Of the six inactivators, only metergoline produced less than a complete inactivation at 1 μM, indicating that it is either less potent than the other five inactivators or producing its inactivation through a distinct and less efficacious mechanism.

To obtain more information on this novel inactivation property of the six drugs, concentration-response curves for h5-HT_7 inactivation were produced (Fig. 2 and Table 1). The h5-HT_7 receptor-expressing HEK cells were first exposed to increasing concentrations of drug for 30 min, followed by three washouts. The cells were then exposed to 10 μM 5-HT for 30 min. Risperidone, 9-OH-risperidone, lisuride, and methiothepin displayed high potencies for producing the inactivation effect, with IC_{50} values of 1.3, 3.9, 1.0, and 3.0 nM, respectively. Bromocryptine and metergoline displayed lower potencies with IC_{50} values of 80 and 321 nM, respectively.

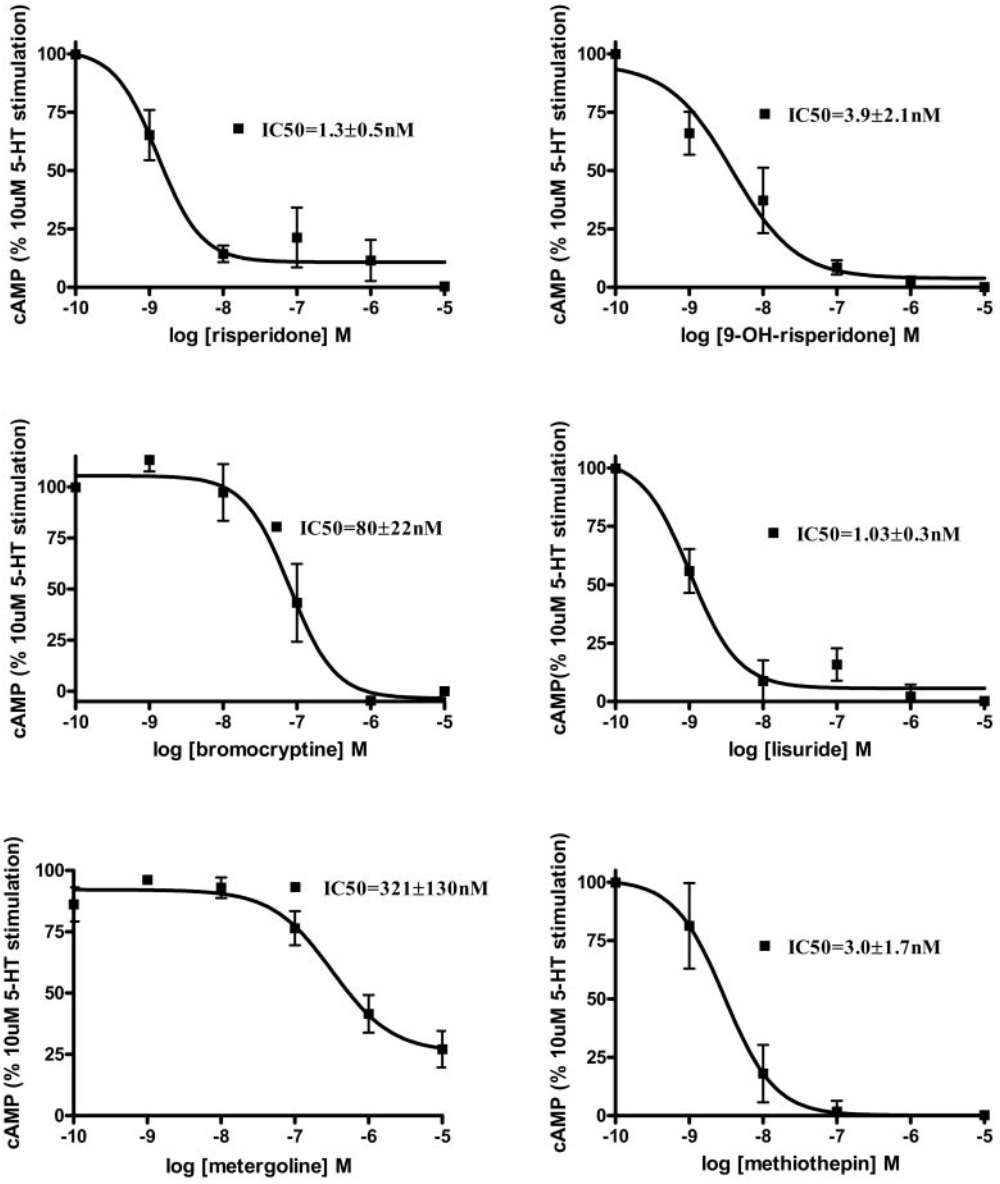

Fig. 2. Concentration-response curves for inactivation of h5-HT_7 receptor-stimulated cAMP production. HEK293 cells expressing h5-HT_7 receptors were suspended in isotonic buffer and exposed to buffer (control) or varying concentrations of the six drugs displaying inactivating properties (see Fig. 1). After the drug washout procedure (see *Materials and Methods*), the cells were exposed to 10 μM 5-HT for 30 min. cAMP levels were determined using the LANCE cAMP Detection kit (Perkin-Elmer). The results are the means ± S.E.M. of three independent experiments performed in triplicate.

Five of the six inactivating drugs were able to fully inactivate the receptor at 10 μM concentrations. Metergoline produced a maximal inhibition of activity of ~75%. The potencies of the inactivators closely corresponded with their affinities for the h5-HT$_7$ receptor with the exception of metergoline. Metergoline is a high-affinity ligand for the h5-HT$_7$ receptor (K_i = 16 nM). Thus, it seems that the potency of inactivation involves more than occupancy of the receptor, possibly involving differing inactivation kinetics for different inactivators (see below).

TABLE 1

Comparison of affinities of the six inactivating drugs for the h5-HT$_7$ receptor in radioligand binding assays with their potencies as insurmountable inhibitors of h5-HT$_7$ receptor binding or inhibitors of h5-HT$_7$ receptor activity

IC_{50} values are reported as the receptors are tested subsequent to the removal of the inactivating drug. Results are the means ± S.E.M. of three independent experiments performed in triplicate.

Drug	h5-HT$_7$ Affinity (Membrane Homogenate Radioligand Binding) (K_i)	Insurmountable Inhibition (IC_{50})	
		h5-HT$_7$ Whole-Cell Binding	h5-HT$_7$-Receptor-Mediated cAMP Accumulation
	nM		*nM*
Risperidone	1.8 ± 0.3	9 ± 4	1.3 ± 0.5
9-OH-Risperidone	10 ± 1.7	5.5 ± 3.6	3.9 ± 2.1
Bromocryptine	143 ± 56	152 ± 52	80 ± 22
Lisuride	0.4 ± 0.2	3.0 ± 1.1	1.0 ± 0.3
Metergoline	16 ± 2	73 ± 22	321 ± 130
Methiothepin	3.0 ± 0.5	9.8 ± 1.3	3.0 ± 1.7

To explore the relationship between receptor occupancy and receptor inactivating activity for the six inactivators, we incubated increasing concentrations of the six inactivators with the h5-HT$_7$ receptor-expressing cells, thoroughly washed the cells, and then measured receptor occupancy using [^{3}H]5-HT to label the receptors (Fig. 3). Although bromocryptine, metergoline, and methiothepin produced a potent and complete inhibition of binding, risperidone and 9-OH-risperidone produced surprising concentration-response curves. Maximal concentrations of risperidone and 9-OH-risperidone inhibited 50% of the receptor binding, despite the previous observation that these drugs potently and completely inactivate the h5-HT$_7$ receptors (Fig. 2). Lisuride pretreatment also produced less than a complete loss of binding sites, inhibiting 82 ± 4% of the binding, which was a significantly different effect from any of the other inactivators (Fig. 3).

To explore the properties of the [^{3}H]5-HT binding remaining after pretreatment with risperidone and 9-OH-risperidone (Fig. 3), [^{3}H]5-HT competition curves (Fig. 4) and saturation analyses (Fig. 5) were performed after drug pretreatment and three washes. As shown in Fig. 4, the pharmacological properties of the remaining specific [^{3}H]5-

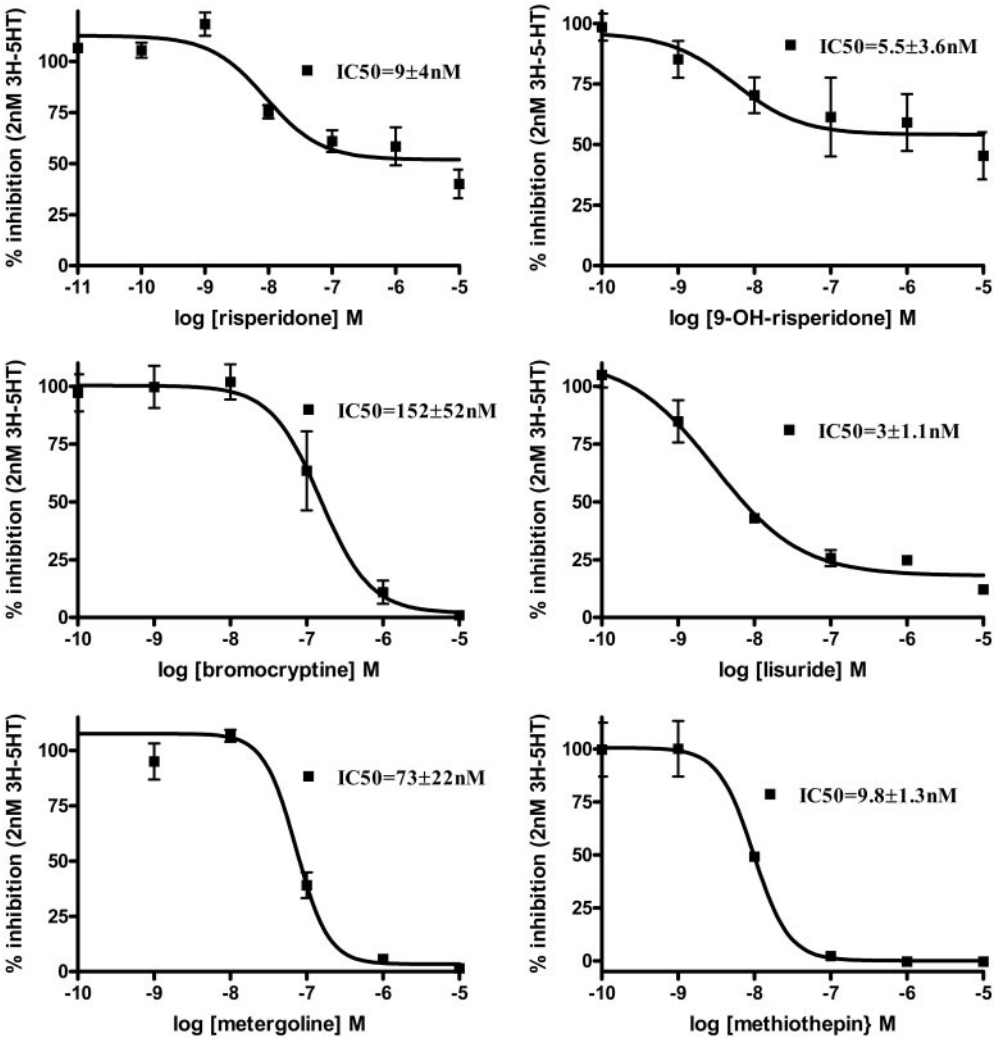

Fig. 3. Concentration-response curves for loss of specific [^{3}H]5-HT binding to h5-HT$_7$ receptor after drug removal. HEK293 cells expressing h5-HT$_7$ receptors were suspended in isotonic buffer and exposed to buffer (control) or varying concentrations of the six drugs displaying inactivating properties (see Fig. 1). After the drug washout procedure (see *Materials and Methods*), the cells were incubated with 2 nM [^{3}H]5-HT in the presence and absence of 10 μM clozapine. The results are the means ± S.E.M. of three independent experiments performed in triplicate.

HT binding is that of the h5-HT_7 receptor, displaying high affinity for the selective 5-HT_7 antagonist SB269970. Saturation analyses of high-affinity [^{3}H]5-HT binding indicate that ~50% of the binding was removed, with no change in affinity for [^{3}H]5-HT (Fig. 5). Thus, pretreatment with risperidone and 9-OH-risperidone can completely inactivate the h5-HT_7 receptor-mediated response but can only irreversibly block 50% of the h5-HT_7 receptors.

Discussion

The effects of risperidone, 9-OH-risperidone, lisuride, bromocryptine, methiothepin, and metergoline on the h5-HT_7 receptors expressed in HEK293 cells display several novel characteristics. Most dramatic is the rapid, potent, and complete inactivation of the receptor after exposure to these drugs (Figs. 1 and 2). Although a majority of the 20 drugs tested to date in this series of studies display classic competitive antagonism, these six drugs clearly produce a drug-receptor interaction that results in an inactivated receptor. In earlier studies with [^{3}H]risperidone, a pseudo-irreversible interaction with the h5-HT_7 receptor was directly demonstrated (Smith et al., 2006). This interaction apparently explains the inactivation effect. It would seem that the five other inactivating drugs produce a similar pseudo-irreversible effect. Because of the lack of radiolabeled forms of these drugs, this effect cannot be directly demonstrated. The structural features of the six inactivating drugs that allow the production of the pseudo-irreversible complex with the h5-HT_7 receptor are not immediately obvious because the six drugs represent diverse chemical families. Risperidone and 9-OH-risperidone (benzisoxazole derivatives) are similar in structure and both produce the inactivating effect. Lisuride, bromocryptine, and metergoline are ergoline derivatives. However LSD, another ergoline derivative, does not produce the inactivating effect (Fig. 1). Methiothepin is a methylpiperazine with no obvious similarities to risperidone or the ergolines. It is possible that computer modeling of the structures might reveal a similar structural motif not obvious in a two-dimensional figure.

It was anticipated that performing radioligand binding studies after exposing the cells to the inactivating ligands would produce potent inhibition of the radioligand binding signal, reflective of the potent inactivating properties of these drugs. However, as shown in Figs. 2 and 3, the inactivating drugs fell into two groups. Methiothepin, metergoline, and bromocryptine produced the anticipated potent and complete inhibition of radioligand binding predicted for drugs pseudo-irreversibly complexed to the h5-HT_7 receptor. Lisuride produced a potent and nearly complete inhibition (Fig. 3). However, risperidone and 9-OH-risperidone produced a puzzling effect. Both drugs potently inhibited 50% of the receptor binding. Thus, 50% of the h5-HT_7 receptors seem to be risperidone- and 9-OH-risperidone-resistant (Figs. 3 and 5). This resistant binding seems to be composed of intact h5-HT_7 receptors, based on the affinity for SB269970 (Fig. 4).

The results in Figs. 2 and 3 indicate that there are at least two slightly different mechanisms of inactivation occurring. Although the six inactivators can inhibit all of the functional activity of h5-HT_7 receptors, as judged by 5-HT-stimulated cAMP production, risperidone and 9-OH-risperidone produce only a 50% loss of [^{3}H]5-HT binding sites. The other four inactivators are efficacious inhibitors of [^{3}H]5-HT specific binding to h5-HT_7 receptors (Fig. 3). These results imply that the six inactivators share the ability to stabilize a conformation of the receptor that inactivates G proteins. This inactivation seems to be very stable, presumably because of the pseudo-irreversible interaction of the inactivators with the h5-HT_7 receptor revealed in a previous publication (Smith et al., 2006). The rationale for the loss of 50% of the binding sites, rather than all of the binding sites, because of the pretreatment with risperidone or 9-OH-risperidone is under investigation. These results imply a possible receptor-receptor interaction modified by risperidone and 9-OH-risperidone. Given the 50% effect observed, the possibility of the involvement of dimerization seems likely. One possibility is that the binding of risperidone or 9-OH-risperidone to one

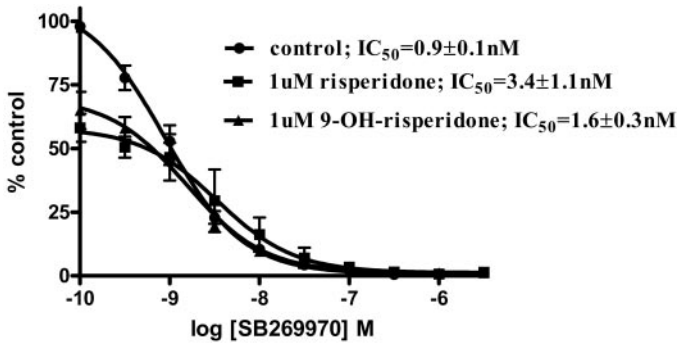

Fig. 4. Affinities of SB-269970 for [^{3}H]5-HT-specific binding after pretreatment with no drug (control), 1 μM risperidone, or 1 μM 9-OH-risperidone. Consistent with Figs. 3 and 5, 50% of the h5-HT_7 receptors are risperidone- and 9-OH-risperidone-resistant. The high affinities displayed by SB269970 indicate the risperidone- and 9-OH-risperidone-resistant radioligand binding signal displays the pharmacological properties of the h5-HT_7 receptor. The results are the means ± S.E.M. of three independent experiments.

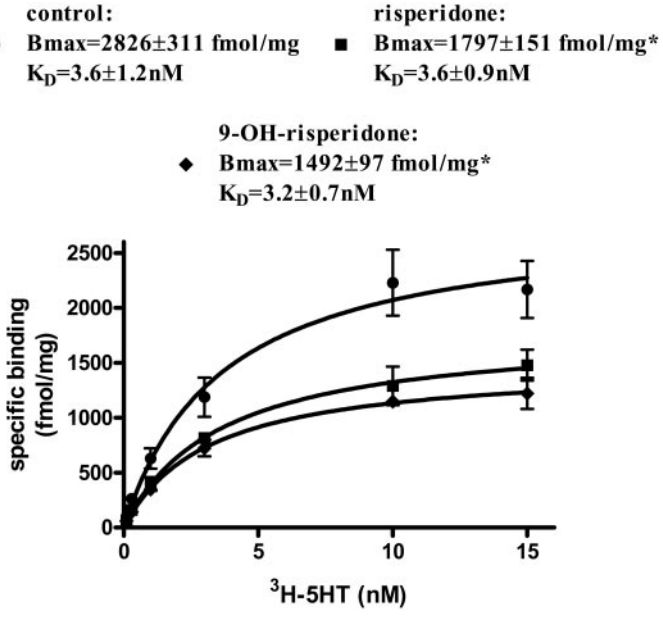

Fig. 5. Saturation analyses of specific [^{3}H]5-HT binding to HEK293 cells expressing the h5-HT_7 receptor after pretreatment with no drug (control), 1 μM risperidone, or 1 μM 9-OH-risperidone. After drug pretreatment and washout procedure, cells were incubated with increasing concentrations of [^{3}H]5-HT in the absence and presence of 10 μM clozapine. The results are the means ± S.E.M. of three independent experiments performed in triplicate. *, $p < 0.001$ versus control B_{max} values.

protomer of a dimer pair produces an allosteric effect on the other protomer. The second protomer would presumably lose its ability to irreversibly bind risperidone or 9-OH-risperidone, thus leaving 50% of the sites available for radiolabeling. This possibility is currently under investigation.

Another surprising result is the relatively low potency of metergoline relative to the other inactivators. Metergoline is a high-affinity ligand (K_i = 16 nM; Table 1) but displayed a relatively low potency inactivation (IC_{50} = 112 nM; Fig. 2). Bromocryptine displayed a lower potency as an inactivator, but it also displays a lower affinity (K_i = 143 nM) than the other inactivators. It may be that each inactivating drug has a different time course for producing the pseudo-irreversible complex and that increasing the incubation time for metergoline will produce a higher potency interaction.

The h5-HT$_7$ receptor has displayed unusual properties since its discovery through homology cloning (Bard et al., 1993; Lovenberg et al., 1993; Shen et al., 1993). The original characterization revealed that this receptor displays high affinities for agonists, whether the radiolabel is an agonist or antagonist ligand. This is contrary to most group 1 GPCRs, which display lower affinities for agonists when antagonist radioligands are used (Lyon et al., 1987). Several studies have indicated that a 5-HT$_7$-receptor/G-protein complex may exist in the absence of an agonist (Krobert et al., 2001; Bruheim et al., 2003; Kvachnina et al., 2005). Of special interest is a report that methiothepin produces an irreversible inhibition of the h5-HT$_7$ receptor (Krobert et al., 2006). However the authors of that article attribute the methiothepin effect to high lipophilicity producing a slow dissociation. The washout studies performed in the studies reported herein remove >90% of the drugs (data not shown). Thus, residual drug is not a likely mechanism for the inability of 10 μM 5-HT to stimulate the h5-HT$_7$ receptor subsequent to exposure to methiothepin or the other inactivating drugs. It is likely that the "persistent" loss of activity and binding sites observed after methiothepin pretreatment (Krobert et al., 2006) was due to the unusual interaction of methiothepin with the h5-HT$_7$ receptor as described herein.

In examining the literature for GPCRs that have been reported to produce some of the novel effects reported herein, a member of the neuropeptide Y receptor family seems to be of interest. The neuropeptide Y receptor family is a member of the type 1 GPCR family and is composed of four receptors: Y1, Y2, Y4, and Y5 (Ouedraogo et al., 2008). Two observations reported for the Y2 receptor are intriguing. Most notably, neuropeptide Y and a small molecular antagonist BIIE0246 react irreversibly with the Y2 receptor in radioligand binding studies (Dautzenberg and Neysari, 2005). BIIE0246 irreversibly inactivates the Y2 receptor in a manner very similar to the inactivators of the h5-HT$_7$ receptor reported herein (Dautzenberg and Neysari, 2005). Another similarity is that unlike the other members of the neuropeptide Y receptor family, the Y2 receptor does not internalize upon agonist stimulation (Dautzenberg and Neysari, 2005). The h5-HT$_7$ receptor also does not internalize in the presence of 10 μM 5-HT, as determined using green fluorescent protein-tagged h5-HT$_7$ receptors to monitor receptor trafficking with confocal microscopy (Smith et al., 2006). It may very well be that there are other members of the type 1 GPCR family that possess the unusual properties demonstrated by the h5-HT$_7$ receptor. However, revealing these properties requires the appropriate drug(s) and the appropriate experiments. It is possible that GPCRs from diverse subgroupings may possess structural components that allow the pseudo-irreversible interaction with endogenous ligands and/or antagonists. Drugs that interact irreversibly with these receptors may produce therapeutic and/or side effects not seen with competitive antagonists.

Results of a study examining 5-HT$_7$ receptor pharmacology produced observations consistent with the results reported herein (Terron, 1997). Canine cerebral artery vasodilation was demonstrated to be mediated by the 5-HT$_7$ receptor through the use of antagonists to reverse 5-HT-mediated vasodilation. Three drugs were found to produce noncompetitive antagonism: risperidone, methiothepin, and lisuride (Terron, 1997). Consistent with this report, we observe irreversible effects with these drugs. It is interesting that the 1997 report found metergoline to be a competitive antagonist (Terron, 1997). Although this is apparently inconsistent with our results, in which metergoline was found to produce an irreversible effect, a closer examination of our data indicates that this is not actually inconsistent. Relative to the other irreversible antagonists, metergoline is a far lower potency irreversible antagonist than expected from its affinity (Fig. 2). The low dosages used in the 1997 study would not be expected to produce the irreversible effect we observed at relatively high concentrations of metergoline.

In summary, the results presented indicate drug-receptor interactions unobserved previously in the monoamine GPCR family. The pseudo-irreversible interaction of a significant population of drugs (~29% of the drugs tested to date) with the receptor, producing a complete inactivation, has not been reported for any GPCR. These results may indicate that there is a novel class of drugs, "GPCR inactivators," sharing the properties of the six drugs described herein. The ability of risperidone and 9-OH-risperidone to completely inhibit functional activity of the h5-HT$_7$ receptor whereas only occluding 50% of the binding sites is also a novel observation. This may involve functional receptor-receptor interactions not studied previously.

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